were graft versus host disease (acute/chronic, grade, day of onset, affected organ), infections, survival, relapse, day of bone marrow regeneration and immunosuppression therapy. We found a statistically significant association between rising levels of HLA-G during transplantation and clinically relevant (grade II-IV) acute GvHD, infectious events after transplantation, in particular fungal infections, and development of chronic GvHD. Our preliminary data shows that sHLA-G molecules are involved in several complications after allogeneic hematopoietic cell transplantation.

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AN IN-VIVO MODEL OF HUMAN T CELL-MEDIATED REJECTION OF ALLOGENEIC MISMATCHED HEMATOPOIETIC CD34 + STEM CELLS USING NOD/SCID Γ^{NULL} (NOG) MICE

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Anti-human CD34+ cell T cell alloreactivity was previously shown in-vitro. In this study we transplanted human CD34+ cells and allogeneic T cells in a NOD/SCID γ^{null} (NOG) mouse model to evaluate the occurrence of stem cell rejection in-vivo. After sublethal irradiation NOG mice received 2×10^5 CB CD34+ cells and allogeneic blood T cells at 1:0 (control), 1:2, or 1:10 CD34+ cell: T cell ratio (n = 5-10 mice per group). Human cell engraftment was assessed in the bone marrow and in the spleen 6 weeks following transplantation. Marrow engraftment of huCD45+ cells was 60 ± 10% in control mice and included B cells (64 ± 4%), CD34+ cells (18 ± 1%), CD33+ myeloid cells (7 ± 1%), CD14+ monocytes (3 ± 1%). In contrast, mice that were transplanted with CD34+ cells and low dose (1:2 ratio) or high dose (1:10 ratio) allo-T cells had only 9 ± 2% and 3 ± 1% huCD45+ cells, respectively, in the bone marrow ($p = 0.01$) and >98% were huCD3 + T cells. Spleen engraftment of huCD45+ cells was lower (25 ± 8%) in control mice (1:0 ratio) as compared to 66 ± 10% and 36 ± 11% in 1:2 and 1:10 groups, respectively ($p = 0.05$). However, also the spleen of mice receiving CD34+ and T cells included >98% CD3 + T cells. Among the T cells, both in the marrow and in the spleen of mice in the 1:2 and 1:10 ratio groups, 60-70% were CD4 + CD8- cells, 22-25% CD8 + CD4- cells, 1-3% CD56+ cells, and 2-5% CD4 + CD25+ cells. Only in mice receiving low doses of T cells, on average 12 ± 6% of the T cells in the bone marrow and spleen were CD4 + CD8+. Mice receiving high doses of T cells had acute xenogeneic GVHD demonstrated by fur changes, reduced survival ($p = 0.02$) and weight loss ($p = 0.0001$) compared to control mice or mice receiving a lower dose of T cells (1:2 ratio). In-vitro mixed leukocyte cultures with irrad CD34+ cells and allogeneic T cell responders (R) at the same ratio as in in-vivo experiments were then performed w/wo the addition of CD4 + CD25+ regulatory T cells (Tregs) at 1:1 or 1:5 Treg: R ratio. Since a 60% T cell suppression was observed only with equal numbers of Tregs and allo-responders, we are currently testing whether auto or allo Tregs may prevent stem cell rejection in NOG mice transplanted with CD34+ cells, allogeneic T cells and Tregs at 1:2:2 or 1:1:2 ratio. NOG mice represent a useful model to study human T cell mediated bone marrow failure or stem cell rejection and will allow us to investigate new strategies of allogeneic transplantation with subsets of T cells or hematopoietic stem cells.

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BRONCHIOLITIS OBLITERANS AFTER ALLOGENEIC STEM CELL TRANSPLANTATION – AN ANALYSIS OF 50 CASES OVER THIRTY YEARS AT A SINGLE BRAZILIAN INSTITUTION

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Introduction: Bronchiolitis obliterans (BO) is a late complication of hematopoietic stem cell transplantation (HSCT) usually associated with chronic graft versus host disease (C-GVHD) and often fatal. We describe here characteristics and clinical course of 50 patients at a brazilian transplant center who developed bronchiolitis obliterans as a manifestation of chronic graft versus host disease.

Patients and Methods: We searched the database and reviewed the medical records of 1645 patients who received HSCT between 1979 and 2009, and identified 50 patients who developed BO, whose clinical features and clinical course were analyzed retrospectively. The diagnosis of BO was determined by either the presence of an obstructive ventilatory defect on a pulmonary function test or a chest CT showing small airways wall thickening or air trapping associated with a typical clinical picture in the context of a patient with C-GVHD. NIH criteria were used to define the severity of C-GVHD.

Results: The prevalence of BO in these 30 years was 3%. Patient characteristics are summarized in Table 1. According to the severity of pulmonary involvement 14 patients (28%) had score 1, 12 (24%) had score 2, and 22 (44%) had score 3. Median time between transplantation and diagnosis of C-GVHD was 138 days (33-3738), while median time between the diagnosis of C-GVHD and the diagnosis of BO was 77 days (0 – 1752). Median time between transplantation and diagnosis of BO was 343 days (38 – 3877). Twenty-nine patients (58%) required second-line treatment due to lack of response. The main secondary treatments used were: thalidomide(11); azathioprine(18), tacrolimus (7); mycophenolate mofetil (7) and photopheresis (1). Median survival for this group was 1637 days (195 – 6102). At the time of this analysis 23 (46%) patients had died from BO related causes.

Conclusion: Bronchiolitis obliterans was a serious late complication, occurring in 3% of patients transplanted in our center over the past 30 years. Mortality rate was high (46%) and most patients (58%) did not respond to primary therapy. Better understanding of the pathophysiology of C-GVHD is necessary for the development of more effective therapeutic tools.

Bronquiolitis Obliterans- Patient Characteristics

CHARACTERISTICS	N = 50
age	25 (2 – 50)
Recipient M / Donor F	20(40%)
Donor and recipient match	
matched	40 (80%)
Source of stem cell	
BM	46 (92%)
other (PB or CB)	4(8%)
Donor	
related	45 (90%)
unrelated	5 (10%)
Diagnosis	
CML	24 (48%)
AML	8 (16%)
ALL	5 (10%)
Fanconi Anemia	5 (10%)
Others	8 (16%)
Chronic GVHD onset	
progressive	19 (38%)
quiescent	19 (38%)
de novo	8 (16%)
after DLI	3 (6%)
Risk characteristics	
platelets less than 100.000/mm3	21 (42%)
>3 organs	27 (54%)
<1500 lymphocytes	39 (78%)
NIH global scoring	
Moderate	14 (28%)
Severe	36 (72%)

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PKC$_\Theta$ IS REQUIRED FOR ALLOREACTIVITY AND GVHD BUT NOT FOR IMMUNE RESPONSES TOWARD LEUKEMIA AND INFECTION IN MICE

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